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(54) Title: SOLID PHASE ASSAY

(57) Abstract: The invention relates to a solid phase assay for the detection of antiphospholipid antibodies, where the assay involves contacting a patient's plasma to an assay strip including a phosphatidylethanolamine (PE) in a non-bilayer phase adhered to a solid support material, then developing the assay strip such that the developed strip can be read for antibody-phospholipid reactions. The invention also relates to an assay strip capable of being used for the detection of anti-phospholipid antibodies, where the assay strip includes a PE in a non-bilayer phase.

SOLID PHASE ASSAY

Field of the Invention

The invention relates to a solid phase assay for the detection of anti-phospholipid antibodies.

Background of the Invention

Lipid supramolecular structure is an important consideration in the etiology of autoimmunity. Lupus anticoagulant activity can be specifically induced in mice by unique phospholipid macromolecular architectures. Rauch, et al., *Proc. Natl. Acad. Sci. USA*, *87*:4112-4114 (1990). Further, human hybridoma and plasma-derived lupus anticoagulant antibodies from patients with systemic lupus erythematosus (SLE) specifically recognize hexagonal (H_{II}), but not bilayer, phase phosphatidylethanolamine (PE). Rauch, et al., *J. Biol. Chem.*, *261*:9672-9677 (1986); Rauch, et al., *Thromb. Haemost.*, *62*:892-896 (1989). This specificity was shown indirectly by the ability of non-bilayer, but not bilayer, phase PE to inhibit the lupus anticoagulant activity of these antibodies in the activated partial thromboplastin time (APTT) assay. Such phase-specific phospholipid inhibition has been recommended as an indirect assay for the clinical detection of lupus antibodies and has been found to be both sensitive and specific. Brandt, et al., *Thromb. Haemost.*, *74*:1185-1190 (1995). However, a simple and direct method for clinical detection of lupus anticoagulant antibodies and other phase-sensitive anti-phospholipid antibodies has not been discovered to date.

Summary of the Invention

Briefly, the invention relates to a solid phase assay for the detection of anti-phospholipid antibodies, where the assay involves contacting a patient's plasma to an assay strip comprising a PE in a non- bilayer phase adhered to a solid support material, then developing the assay strip such that the developed strip can be read for antibody-phospholipid reactions. Preferably, the PE is dioleoylphosphatidylethanolamine (DOPE) or egg phosphatidylethanolamine (egg PE), and the solid support material is

nitrocellulose paper. Preferably, the assay strip is developed via immunodot blot analysis. Preferably, the solid phase assay is utilized for the detection of lupus anticoagulant antibodies.

The invention also relates to an assay strip capable of being used for the detection of anti-phospholipid antibodies, where the assay strip comprises a PE in a non-bilayer phase, preferably DOPE or egg PE, adhered to a solid support material, preferably nitrocellulose paper.

Brief Description of the Drawings

Fig. 1 shows the ^{31}P NMR spectra (preformed at 37°C) of DOPE bound to nitrocellulose paper (top) and in buffer suspension (bottom).

Fig. 2 shows the ^{31}P NMR spectra (preformed at 37°C) of egg PE bound to nitrocellulose paper (top) and in buffer suspension (bottom).

Fig. 3 shows the ^{31}P NMR spectra (preformed at 37°C) of palmitoyloleoylphosphatidylethanolamine (POPE) bound to nitrocellulose paper (top) and in buffer suspension (bottom).

Fig. 4 shows the ^{31}P NMR spectra (preformed at 37°C) of dioleoylphosphatidylserine (DOPS) bound to nitrocellulose paper (top) and in buffer suspension (bottom).

Fig. 5 shows the ^{31}P NMR spectra (preformed at 37°C) of bovine heart cardiolipin (CL) bound to nitrocellulose paper (top) and in buffer suspension (bottom).

Fig. 6 shows the ^{31}P NMR spectra (preformed at 37°C) of dioleoylphosphatidylcholine (DOPC) bound to nitrocellulose paper (top) and in buffer suspension (bottom).

Fig. 7 shows the immunodot blot analysis of the reactivity of representative human hybridoma antibodies with non-bilayer and bilayer phospholipids. Strips of nitrocellulose paper, spotted with phospholipids (DOPE, egg PE, DOPS, CL, DOPC and POPE) in $CHCl_3$, were incubated with human hybridoma antibody culture supernatants: B3123 (strip 1), 1420 (strip 2), 9703 (strip 3), 1407 (strip 4) and 1305

(strip 5), where B3123 was negative for lupus anticoagulant activity, while the other four antibodies were positive for lupus anticoagulant activity. The center of each phospholipid spot is indicated by a dot, made in pencil prior to spotting the lipids. Antibody binding was detected with HRP-conjugated goat anti-human immunoglobulin and 3,3'-diaminobenzidine. As the blots were read wet for scoring (positive or negative) in Table 2, but photographed dry, weakly positive spots may appear to be negative in Fig. 7.

Detailed Description of the Invention

The invention is a method and apparatus for detecting the presence of phase-sensitive anti-phospholipid antibodies in blood plasma. The invention exploits the discovery of direct, phase-sensitive antibody-phospholipid binding to provide a simple yet effectively sensitive assay.

Specifically, the invention is a solid phase assay and an associated assay strip. The solid phase assay involves contacting plasma from any animal with an assay strip comprising a non-bilayer phase PE adhered to a solid support material, then developing the assay strip so that the developed strip can be read for antibody-phospholipid interactions. The PE can be any hexagonal-phase favoring PE to which a phase-sensitive anti-phospholipid antibody preferentially binds, and is preferably DOPE or egg PE, and more preferably DOPE.

The size, shape, configuration and composition of the solid support material are not particularly limited, so long as the PE can be adhered to the support in a non-bilayer phase, and the resulting assay strip can be contacted with plasma and developed to reveal antibody-phospholipid interactions. Preferably, the solid support material is nitrocellulose paper.

Any developing technique that reveals the binding of the phase-sensitive anti-phospholipid antibodies to the non-bilayer phase PE can be used in the invention. Preferably, an immunodot blot analysis technique is used to develop the assay strip.

The invention will now be described through specific examples. The examples involve particular embodiments of the invention and are intended to be illustrative. The examples are not intended to limit the scope of the invention defined in the claims.

Materials and Methods Used in the Examples

Phospholipids

Bovine heart cardiolipin (CL), dioleoylphosphatidylcholine (DOPC), DOPE, dioleoylphosphatidylserine (DOPS), egg PE and palmitoyloleoylphosphatidylethanolamine (POPE) were purchased from Avanti Polar Lipids (Birmingham, Alabama) and stored either lyophilized or in chloroform ($CHCl_3$) at -70°C. These phospholipids were used without further purification.

Production of Human-Human Hybridoma Antibodies

Human-human hybridoma lupus anticoagulant antibodies (except 4003, which was derived from a normal individual) were produced from peripheral blood lymphocytes of patients with SLE, most with circulating lupus anticoagulant antibodies, as described in Rauch, et al., *J. Biol. Chem.*, *261*:9672-9677 (1986) and Massicotte, et al., *Hybridoma*, *3*:215-222 (1984), the pertinent portions of which are incorporated herein by reference. Hybridoma antibodies without lupus anticoagulant activity were derived from the fusion of lymphocytes from normal individuals of patients with SLE or rheumatoid arthritis. All hybridoma antibodies were IgM immunoglobulins, as determined by a solid phase enzyme-linked immunoassay (ELISA) using commercially available reagents.

Detection of Hybridoma Lupus Anticoagulant Activity

Human hybridoma supernatants (in RPMI-based culture medium, containing 15% fetal bovine serum) were tested and defined as having lupus anticoagulant

activity, using a dilute activated partial thromboplastin time (APTT) assay as described in Rauch, et al., *J. Biol. Chem.*, *261*:9672-9677 (1986) and Rauch, et al., *Thromb. Haemost.*, *62*:892-896 (1989), the pertinent portions of which are incorporated herein by reference. Most of the lupus anticoagulant negative antibodies were negative by ELISA for all autoantigens tested, but some were positive for other autoantibody activities (e.g., B122 was highly polyreactive and reacted with denatured DNA, CL, PE, and platelets by ELISA). See Rioux, et al., *Molec. Immunol.*, *32*:683-696 (1995), the pertinent portions of which are incorporated by reference.

Immunodot Blot Analysis of Hybridoma Antibodies

One µl of a 20 mg/ml solution of phospholipid in chloroform was spotted on 4 mm x 100 mm strips of 0.1 µm nitrocellulose paper (Schleicher and Schuell, Keene, New Hampshire), allowed to dry, and the strips were blocked with 0.01 M Tris-buffered saline, pH 7.4 (TBS) containing 0.5% casein for 1 hour at 37°C. After 5 washes with TBS, hybridoma supernatants (0.5 ml) were applied to the strips and incubated for 120 minutes at 37°C. Following 5 washes with TBS, 1.0 ml of horseradish peroxidase (HRP)-conjugated goat anti-human immunoglobulin (Tago, Burlingame, California) diluted 1/500 in PBS-0.5% casein was added and incubated for 75 minutes at 37°C. The strips were then washed twice with TBS, developed with 3,3'-diaminobenzidine, washed twice with distilled water, and read immediately (wet) or photographed later (dry). Reactions were found to be strongest and most specific when the strips were read immediately after developing, while they were still wet. Positive reactions faded slightly and negative reactions became slightly positive when the strips dried.

^{31}P Nuclear Magnetic Resonance (^{31}P NMR) Analysis of Phospholipid Structure

^{31}P NMR analysis was performed on each phospholipid, both as a dispersion in 20 mM Hepes buffer (150 mM NaCl, pH 7.5) and bound to nitrocellulose paper. The ^{31}P NMR spectra were acquired on a Bruker AM-360 spectrometer operating at 145.8

MHz (8.5 Tesla) on non-spinning samples with a 10 mm double resonance probe. Bloch decays were obtained with a 90° pulse, scalar multipulse proton decoupling during data acquisition, and 6 second interpulse spacings. Approximately 30,000 transients were averaged using a 25 kHz spectral window and 4K data points. An exponential filter of 100 Hz was applied to the decay prior to transformation. The temperature controller was calibrated with ethylene glycol. Samples were allowed to equilibrate at the desired temperature for 20 minutes prior to acquisition. The resonance of small unilamellar vesicles of egg lecithin was used as an external chemical shift reference (0 ppm).

Phospholipid suspensions were prepared by evaporating 50 mg of phospholipid from chloroform in a round-bottom glass tube using a Rotovapor apparatus (Buchi, Flawil, Switzerland) under vacuum. The dried lipids were gassed with dry nitrogen and hydrated at a temperature above their transition temperature for 1 hour. Phosphate concentrations were determined by the method of Bartlett. See Bartlett, G.R., *J. Biol. Chem.*, *234*:466-468 (1959), the pertinent portions of which are incorporated herein by reference.

Twenty mg of phospholipids were spotted in chloroform on 80 mm x 120 mm sheets of 0.1 µm nitrocellulose paper (Schleicher and Schuell, Keene, New Hampshire) using a Hamilton syringe with a repeating dispenser (Hamilton Co., Reno, Nevada). Five hundred spots of 2 µl each of a 20 mg/ml solution of phospholipid in chloroform were used to cover each sheet of nitrocellulose paper. The sheets were air-dried and incubated with 20 ml of TBS containing 0.5% bovine serum albumin (BSA) for 1 hour at 37°C. As casein is a phosphorylated protein, it could not be used for blocking in the ^{31}P NMR experiments and so, was replaced by BSA. However, casein continued to be used for the immunodot blot experiments, as it was superior to BSA in blocking nonspecific binding of immunoglobulins to the nitrocellulose paper. Following the 1 hour incubation, the sheets were washed with TBS and examined by ^{31}P NMR spectroscopy, by inserting the crumpled, disoriented nitrocellulose paper into

the NMR tube so that it was completely contained within the probe coil. The nitrocellulose paper in the NMR tube was hydrated with 1.2 ml of TBS buffer.

To ensure that phospholipid was not washed off the nitrocellulose paper during the blocking and washing steps, small pieces of nitrocellulose paper spotted with 2 µl of each phospholipid in chloroform were assayed for lipid phosphate content both prior to and following the blocking and washing steps. The pieces of nitrocellulose paper were dissolved in 0.4 ml of perchloric acid, which was directly assayed for phosphate content by the method of Bartlett. See Bartlett, G.R., *J. Biol. Chem.*, *234*:466-468 (1959), the pertinent portions of which are incorporated herein by reference.

Example 1 - ^{31}P NMR Analysis of Phospholipid Structure

In order to ensure that the applied phospholipids remained bound to the nitrocellulose paper in the presence of buffer, the amount of phosphate bound to the paper was quantitated before and after incubation with buffer. As nitrocellulose paper is soluble in perchloric acid, it was dissolved and directly assayed in the Bartlett assay. Table 1 shows the µmoles of phosphate bound to the nitrocellulose before and after 1 hour incubation with blocking buffer and after 5 washes with buffer. Phosphate values of the phospholipids used for spotting were virtually identical before and after the buffer incubation and washes. In fact, most samples showed a slightly higher value after blocking, but this was not significant as it was less than or equal to the variability between the duplicates (+ 0.005 µmoles) in the assay procedure. Nitrocellulose paper with no phospholipid was negative for phosphate, indicating that the phosphate values were truly representative of phospholipid bound to the paper and not the paper or blocking protein.

Table 1. Quantitative analysis of phospholipids bound to nitrocellulose paper

Phospholipid	µmoles Phosphate[a]	
	Pre-Blocking /Washing	Post-Blocking/Washing
None ($CHCl_3$)	0	0
DOPE	0.023	0.026[b]
Egg PE	0.023	0.028
DOPS	0.042	0.046
CL	0.027	0.033
DOPC	0.024	0.025
POPE	0.024	0.025

[a]Nitrocellulose paper spotted with phospholipid was assayed for phosphate content before and after incubation with blocking buffer (TBS containing 0.5% BSA) for 1 hour at 37°C and 5 washes with TBS. The percent (%) retained on the paper following the blocking and washing steps was calculated as follows:

$$\frac{\text{µmoles phosphate (post-blocking/washing)}}{\text{µmoles phosphate (pre-blocking/washing)}} \times 100\%$$

[b]slight increase is not significant as the variability between duplicates in the assay is ±0.005 µmoles

^{31}P NMR was used to determine the structural organization of the various phospholipids in suspension and bound to nitrocellulose paper. Upon hydration, bilayer forming phospholipids give rise to spectra characterized by a low field shoulder and high forming phospholipids give rise to spectra characterized by a low field shoulder and high field peak, whereas phospholipids arranged in a non-bilayer, H_{II} phase give rise to spectra with reversed asymmetry, narrower by a factor of two. See Cullis, et al., *Biochim Biophys Acta*, *559*:399-420 (1979), the pertinent portions of which are incorporated herein by reference. It can be seen from Figs. 1-6 that, in suspension, DOPE and egg PE were organized in a H_{II} phase at 37°C, whereas POPE, CL, DOPS and DOPC were arranged in liquid crystalline bilayers. These results are in excellent agreement with previous NMR characterization of these phospholipids. See Cullis, et al., *Biochim Biophys Acta*, *559*:399-420 (1979); Cullis, et al., *Biochim*

Biophys Acta, *513*:31-42 (1978); and Dekker, et al., *Chem. Phys. Lipids*, *33*:93-106 (1983), the pertinent portions of which are incorporated herein by reference.

The bilayer preferring phospholipids (POPE, DOPS, CL and DOPC) formed bilayer structures when hydrated on the nitrocellulose paper. In all cases, the spectral linewidths were narrower: the high field peak shifted downfield and the low field shoulder was shifted upfield. The linewidth or chemical shift anisotropy (CSA) of phospholipids arranged in bilayers is a function of vesicle size (the CSA decreases with decreasing vesicle size) and the reorientation rate of the phospholipid in a vesicle, which is descried by a correlation time, τ_c (the CSA decreases with shorter τ_c). See Burnell, et al., *Biochim Biophys Acta*, *603*:63-69 (1980), the pertinent portions of which are incorporated herein by reference. Since vesicles were not freely tumbling in solution, but lipid was tightly associated with the nitrocellulose paper, the spectral narrowing observed was most likely due to a decrease in τ_c.

In the case of the H_{II} forming phospholipids, DOPE and egg PE, association with the nitrocellulose paper produced spectra that contained a small H_{II} spectral component but mostly a broad, isotropic signal. It is possible, but unlikely, that on solid support, these lipids formed very short hexagonal tubes that were motionally averaged by tumbling. A more likely explanation for the data is that an extended, non-bilayer cubic phase or inverted cylinders sandwiched between lipid monolayers were formed on the nitrocellulose support. The cubic phase has been described as being an intermediate in the bilayer to H_{II} transition for a number of phospholipids; in fact, some lipids undergo bilayer to H_{II} cubic phase transitions. See Tilcock, et al., *Biochemistry*, *21*:4596-4601 (1982), the pertinent portions of which are incorporated herein by reference. Lipids in the cubic phase would be arranged in highly curved structures, similar to lipids residing in the highly curved monolayer surrounding the inverted cylinders comprising the H_{II} phase. Similarly, the monolayer of phospholipid covering extended, inverted cylinders would also likely be highly curved, giving rise to isotropic motional averaging and spectral narrowing. While the exact organization

of DOPE and egg PE on the nitrocellulose paper is unknown, these lipids clearly were not arranged in liquid crystalline bilayers.

Example 2 – Immunodot Blot Analysis of Hybridoma Antibodies

Table 2 shows the results of the immunodot blot analysis of 33 human hybridoma antibodies, of which 16 had lupus anticoagulant activity and 17 did not. The reaction on a panel of seven phospholipids, including DOPE, egg PE, DOPS, CL, DOPC, and POPE, and chloroform ($CHCl_3$) alone is shown for each antibody. The results are summarized in Table 3, which indicates the numbers of lupus anticoagulant positive and lupus anticoagulant negative antibodies reactive with each phospholipid. Of the 16 lupus anticoagulant positive antibodies, 12 (75%) reacted with DOPE, 10 (62%) with egg PE, 7 (44%) with DOPS, 10 (62%) with CL, 2 (12%) with DOPC, and 2 (12%) with POPE. Only three lupus anticoagulant antibodies did not react with any of the phospholipids tested. In contrast, of 17 lupus anticoagulant negative hybridoma antibodies tested, none reacted with DOPE, egg PE, DOPS, DOPC, or POPE. One lupus anticoagulant negative antibody (hybridoma antibody 122) reacted with CL. None of the antibodies tested reacted with chloroform alone.

Table 2. Immunodot blot analysis of human hybridoma antibodies with and without lupus anticoagulant (LA) activity on non-bilayer and bilayer phospholipids

Antibody	$CHCl_3$	DOPE	Egg PE	DOPS	CL	DOPC	POPE
LA+[a]							
1420	-[b]	+	+	-	+	-	-
9703	-	+	+	+	+	+	+
9200	-	+	-	-	+	-	-
9700	-	+	+	-	+	-	-
9705	-	-	-	-	-	-	-
1407	-	+	+	+	+	-	-
1305	-	+	+	-	+	+	-
1206-1	-	+	+	+	+	-	-
4003	-	+	+	+	-	-	+
1104	-	-	-	-	-	-	-
1401	-	-	-	+	-	-	-
1406	-	+	+	+	+	-	-
9706	-	+	+	-	+	-	-
9604	-	+	+	+	+	-	-
1410	-	+	-	-	-	-	-
9603	-	-	-	-	-	-	-
LA-[a]							
1009	-	-	-	-	-	-	-
1000	-	-	-	-	-	-	-
B122	-	-	-	-	+	-	-
B3018	-	-	-	-	-	-	-
B3109	-	-	-	-	-	-	-
B3702	-	-	-	-	-	-	-
B3123	-	-	-	-	-	-	-
B3301	-	-	-	-	-	-	-
B3700	-	-	-	-	-	-	-
B700	-	-	-	-	-	-	-
B3131	-	-	-	-	-	-	-
B3134	-	-	-	-	-	-	-
B3703	-	-	-	-	-	-	-
B3206	-	-	-	-	-	-	-
B3208	-	-	-	-	-	-	-
B3600	-	-	-	-	-	-	-
B3500	-	-	-	-	-	-	-

[a]LA+ and LA- represent antibodies with and without lupus anticoagulant activity, respectively.

[b]Reaction (negative or positive), which indicates the amount of HRP-conjugated goat anti-human antibody bound, was read while the dot blot was still wet.

Table 3. A comparison of hybridoma antibodies with and without lupus anticoagulant activity by immunodot blot analysis

Lupus Anticoagulant Activity	Reactivity on immunodot blot with:						
	$CHCl_3$	DOPE	Egg PE	DOPS	CL	DOPC	POPE
Positive N=16	0 (0%)	12 (75%)	10 (62%)	7 (44%)	10 (62%)	2 (12%)	2 (12%)
Negative N=17	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (6%)	0 (0%)	0 (0%)

All of the 10 lupus anticoagulant antibodies that reacted with egg PE also reacted with synthetic DOPE (Table 2). Two additional lupus anticoagulant antibodies (9200 and 1410) were detected with DOPE. The majority (10/12) of the antibodies reactive with DOPE did not react with POPE. Of the two that did react with POPE, one antibody (9703) also reacted very strongly with DOPC, suggesting that this antibody recognizes an epitope both on DOPC and POPE, as well as epitopes present on H_{II} phase DOPE and PE. The other POPE reactive antibody (4003) did not react with DOPC, but reacted strongly with both H_{II} and lamellar forms of PE.

Interestingly, the 10 lupus anticoagulant antibodies that reacted with CL also reacted with DOPE. Similarly, all but one of the seven lupus anticoagulant antibodies reactive with DOPS were positive on DOPE. This antibody (1401) reacted with DOPS only. Representative dot blots of one antibody without lupus anticoagulant activity (3132) and four antibodies with lupus anticoagulant anticoagulant activity (1420, 9703, 1407 and 1305) are shown in Fig. 7. There was little or no reactivity of the lupus anticoagulant negative antibody with any of the phospholipids. In contrast, all four of the lupus anticoagulant positive antibodies demonstrated clear reactivity with DOPE and egg PE, and most showed visible reactivity with other phospholipids. Three of the four lupus anticoagulant antibodies did not react with POPE, and are representative of the majority of the lupus anticoagulant antibodies shown in Tables 2 and 3. On the other hand, lupus anticoagulant antibody 9703 demonstrated significant binding to POPE and unusually strong reactivity with DOPE, egg PE and DOPC. Each antibody exhibited an individual binding profile. This is particularly well

exemplified by antibodies 1420 and 1407, which showed similar binding to DOPE and egg PE, but differed in their reactivity to DOPS and CL.

Conclusion

The present invention relates to a new phase-specific assay for the detection of anti-phospholipid antibodies. Our invention is based on our finding that hybridoma lupus anticoagulant antibodies can distinguish between different structural forms of PE in a direct-binding solid phase assay. The invention can be used to accurately quantitate both phospholipid and bound antibody, and by using specifically labeled markers, to determine stoichiometric relationships. Bound antibody can also be isolated and analyzed, but equally important, issues of antibody specificity can be decisively resolved. The structurally-sensitive solid phase assay we have discovered utilizes the structural specificity exhibited by lupus anticoagulant antibodies and other anti-phospholipid antibodies. We believe that this phase sensitive solid phase assay may also provide the means to detect different classes of anti-CL antibodies.

Though the invention has been described with reference to specific embodiments detailed herein, it is understood that these are intended to illustrate the invention and that the invention is not necessarily limited thereto. Modifications and variations will be apparent from the disclosure and may be resorted to without departing form the spirit of the invention as understood by those of ordinary skill in the art. Accordingly, such variations and modifications are considered to be within the purview and scope of the invention and the following claims.

What is claimed is:

1. A solid phase assay for the detection of phase-sensitive anti-phospholipid antibodies, said assay comprising:

a) contacting plasma to an assay strip, the assay strip comprising a non-bilayer phase phosphatidylethanolamine adhered to a solid support material; and

b) developing the assay strip such that the assay strip can be read for antibody-phospholipid reactions.

2. The solid phase assay of claim 1, wherein the phosphatidylethanolamine is selected from the group consisting of dioleoylphosphatidylethanolamine or egg phosphatidylethanolamine.

3. The solid phase assay of claim 2, wherein the solid support material is nitrocellulose paper.

4. The solid phase assay of claim 3, wherein the developing is accomplished via an immunodot blot technique.

5. The solid phase assay of claim 4, wherein the phosphatidylethanolamine is dioleoylphosphatidylethanolamine.

6. A solid phase assay for the detection of lupus anticoagulant antibodies, said assay comprising:

a) contacting plasma to an assay strip, the assay strip comprising a non-bilayer phase phosphatidylethanolamine adhered to a solid support material; and

b) developing the assay strip such that the assay strip can be read for antibody-phospholipid reactions.

7. The solid phase assay of claim 6, wherein the phosphatidylethanolamine is selected from the group consisting of dioleoylphosphatidylethanolamine or egg phosphatidylethanolamine.

8. The solid phase assay of claim 7, wherein the solid support material is nitrocellulose paper.

9. The solid phase assay of claim 8, wherein the developing is accomplished via an immunodot blot technique.

10. The solid phase assay of claim 9, wherein the phosphatidylethanolamine is dioleoylphosphatidylethanolamine.

11. An assay strip capable of being used for the detection of anti-phospholipid antibodies, the assay strip comprising a non-bilayer phase phosphatidylethanolamine adhered to a solid support material.

12. The assay strip of claim 11, wherein the phosphatidylethanolamine is selected from the group consisting of dioleoylphosphatidylethanolamine or egg phosphatidylethanolamine.

13. The assay strip of claim 12, wherein the solid support material is nitrocellulose paper.

14. The assay strip of claim 13, wherein the phosphatidylethanolamine is dioleoylphosphatidylethanolamine.

15. The assay strip of claim 14, wherein the anti-phospholipid antibodies are lupus anticoagulant antibodies.

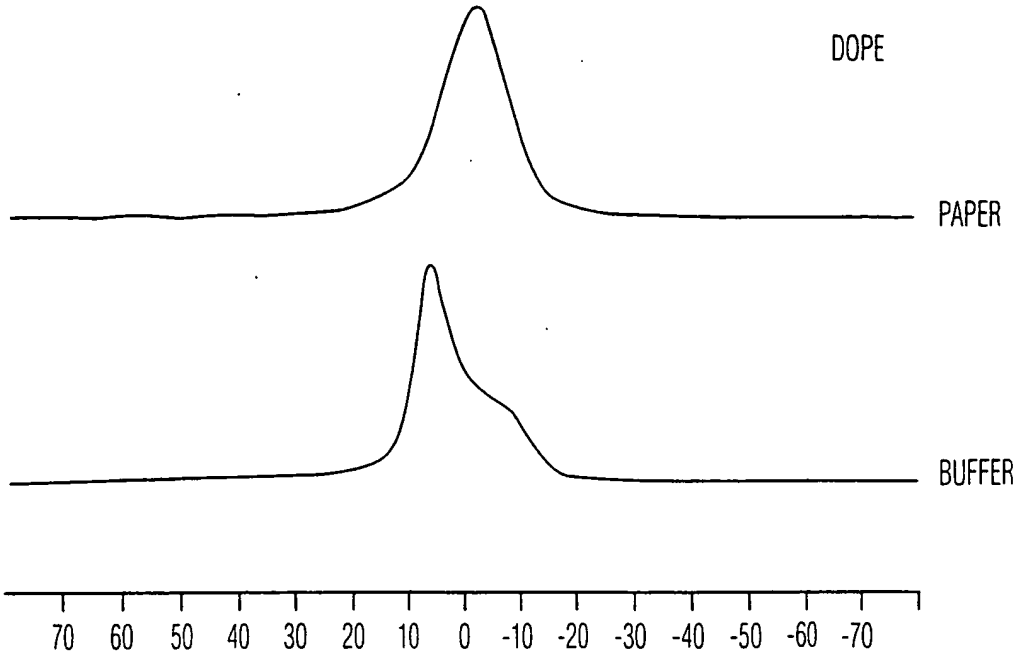

FIG. 1

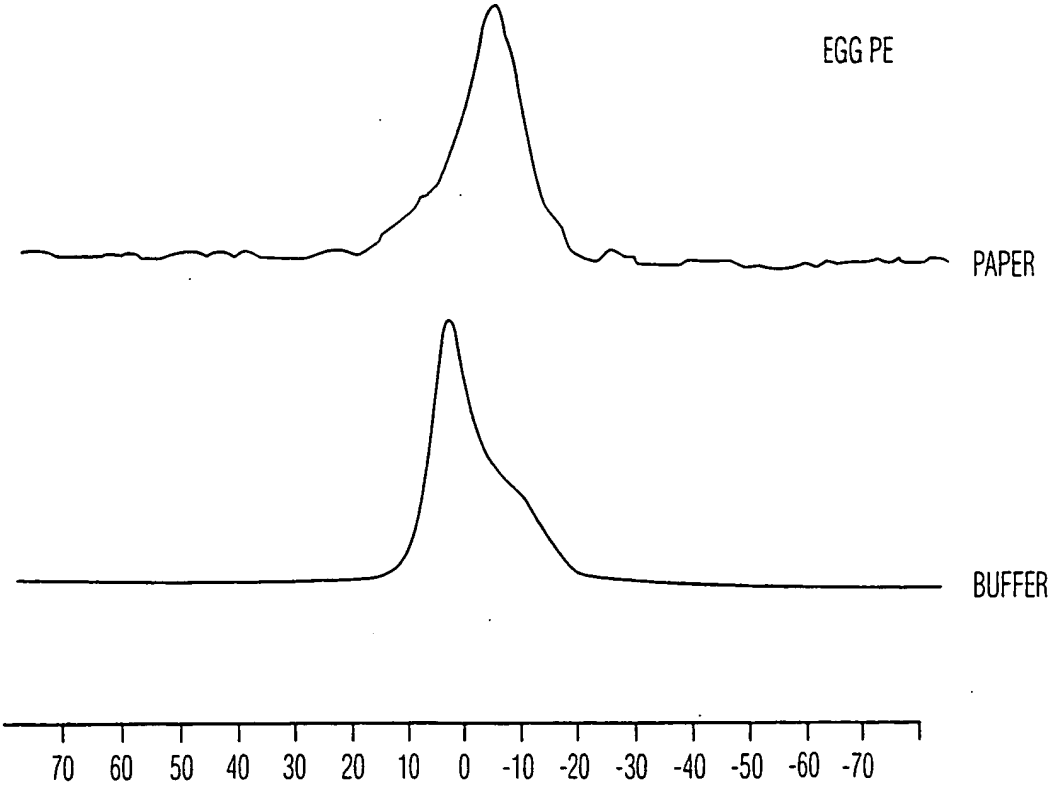

FIG. 2

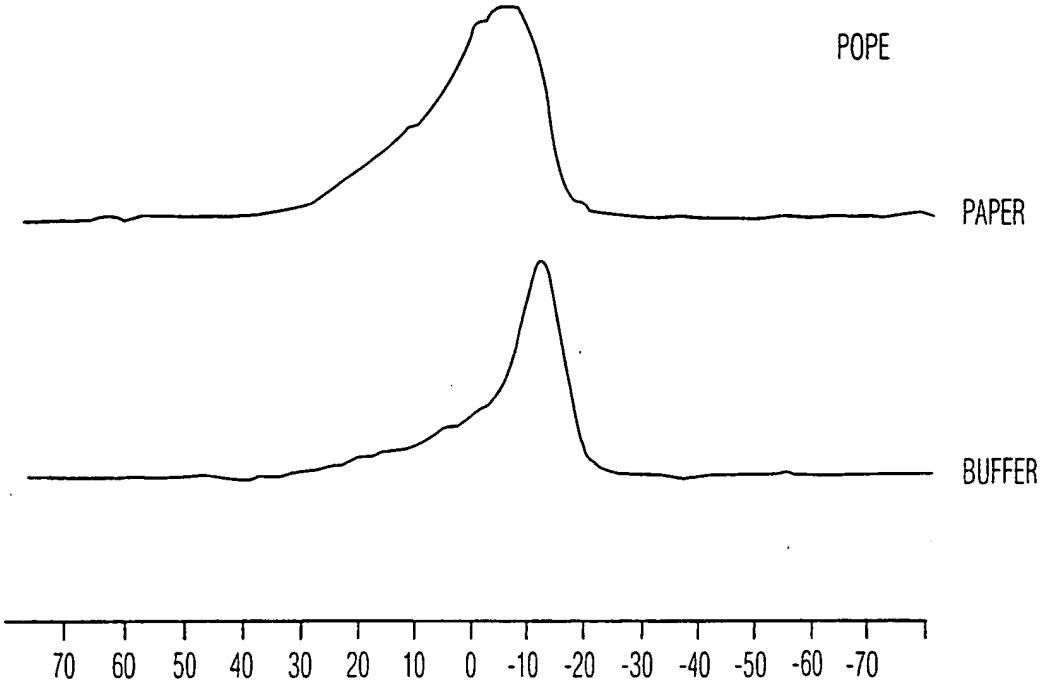

FIG. 3

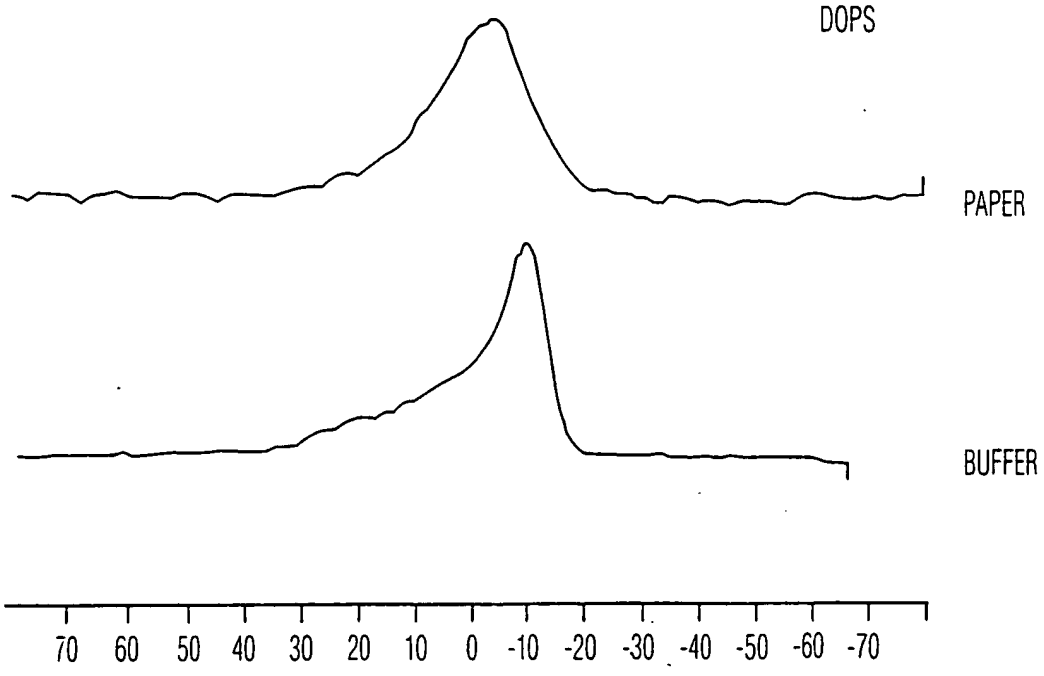

FIG. 4

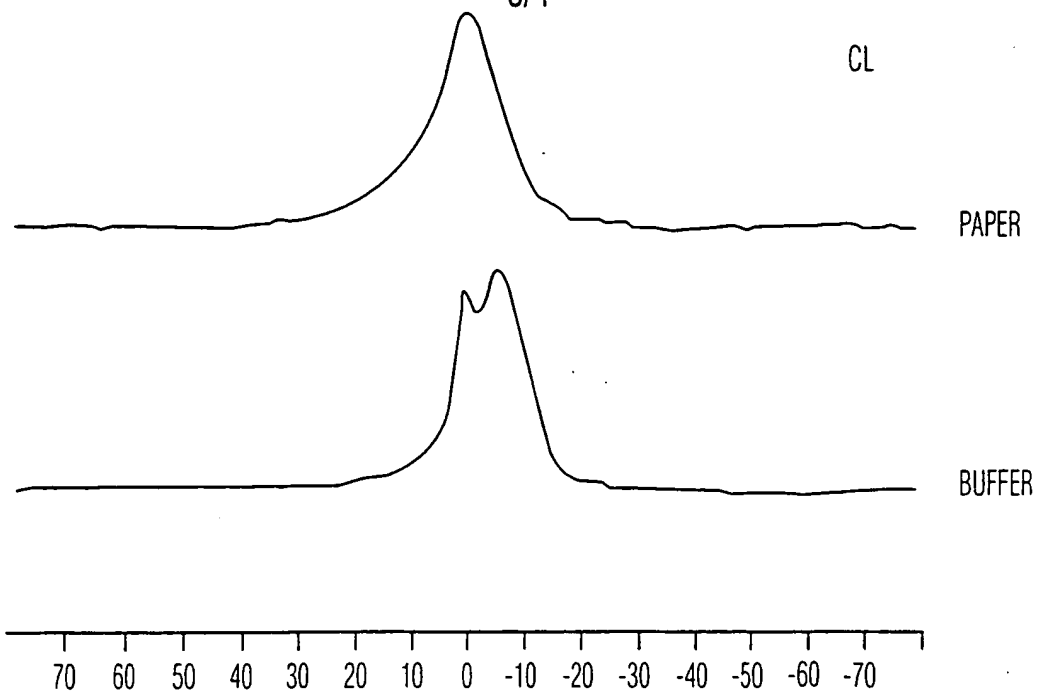

FIG. 5

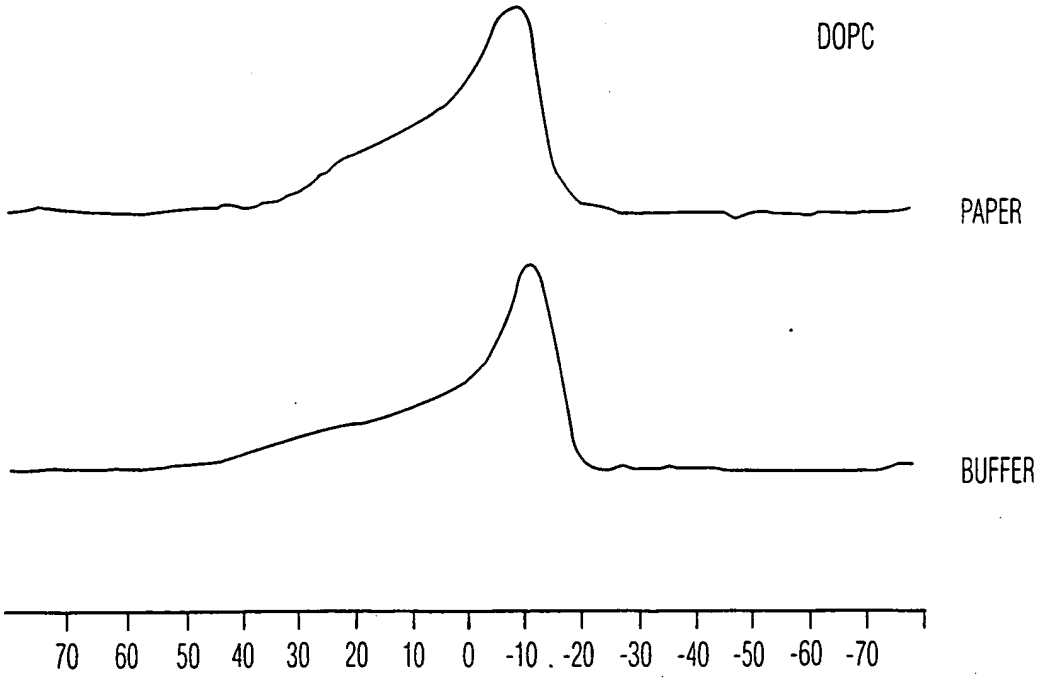

FIG. 6

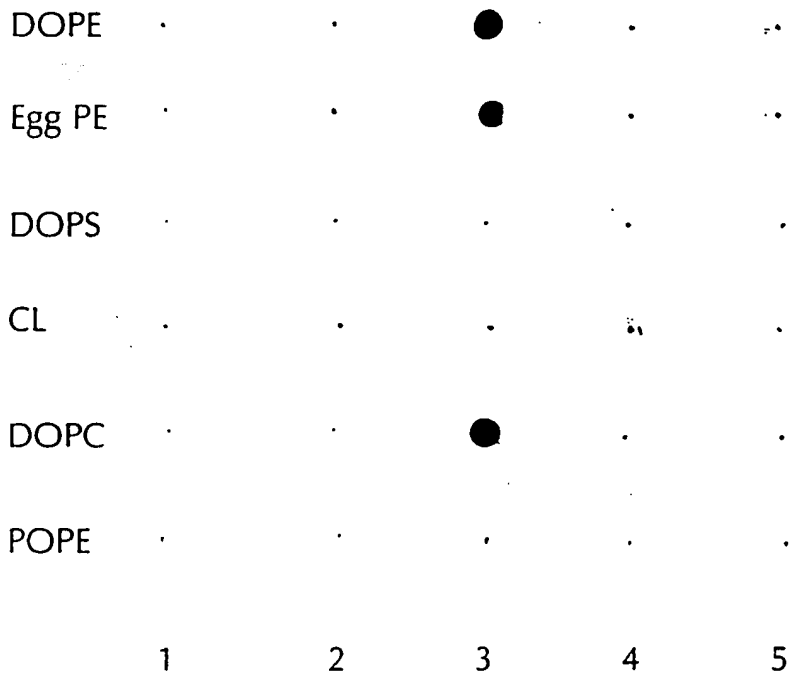

FIG. 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/26067

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 35/16; C12N 11/02; C12Q 1/00; C12M 1/34; G01N 1/18, 33/53, 33/92, 33/543, 33/549, 33/551, 33/573

US CL : 424/530; 435/4, 7.1, 7.2, 7.4, 7.8, 7.92-7.95, 174-182, 287.1, 287.2, 805; 436/71, 178, 518, 524-535, 829

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/530; 435/4, 7.1, 7.2, 7.4, 7.8, 7.92-7.95, 174-182, 287.1, 287.2, 805; 436/71, 178, 518, 524-535, 829

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EAST, STN

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	US 5,972,595 A (KASILA et al) 26 October 1999, whole document.	1-15
A	US 5,776,487 A (MAXFIELD WILSON et al) 07 July 1998, whole document..	1-15
Y	US 4,708,933 A (HUANG et al) 24 November 1987 , whole document..	1-15
Y	US 5,043,164 A (HUANG et al) 27 August 1991 , whole document.	1-15
Y	US 5,506,110 A (MATSUURA et al) 09 April 1996 , whole document.	1-15
X	US 4,564,599 A (JANOFF et al.) 14 January 1986, whole document.	1-15
X	US 4,666,831 A (JANOFF et al) 19 May 1987 , whole document..	1-15

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 October 2000 (27.10.2000)	27 DEC 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Kartic Padmanabhan Telephone No. 703-308-0196

Form PCT/ISA/210 (second sheet) (July 1998)